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Research Notes: Heat Injury Tests as a Screening Tool for Heat Tolerance in Soybeans

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1) Heat injury tests as a screening tool for heat tolerance in soybeans.

One of the objectives of the soybean physiology-breeding program at Nebraska is the evaluation of various procedures used for measuring the response of soybeans to heat and drought stress. We are primarily interested in techniques amenable to large-scale screening of soybean populations or germplasm, so that heat or drought tolerant genotypes can be identified and used in our breeding program. Consequently, the important considerations are rapidity in sampling, simplicity in equipment and procedural requirements, and detection of genotypic (rather than environmental) differences.

One technique that we are currently evaluating appears to hold some promise as a screening tool. This technique has been used successfully in the evaluation of sorghum cultivars for heat and drought tolerance (Sullivan, 1972). The technique is based on the observation that when leaf tissue is injured by high temperatures or low humidities, electrolytes diffuse out of the injured cells. If this tissue is bathed in deionized water, the amount of electrolyte "leakage" (which is dependent upon the degree of induced injury) can be determined by measuring the electrical conductivity of the water. Sullivan (1971) has observed that the results of this test agree very well with the heat or dessication tolerance of the intact sorghum plant. Injury can be induced by either elevated temperatures or specific low relative humidities (to produce dessication). However, dessication injury tests are more time-consuming, tedious, and sometimes difficult to control when compared with heat injury tests.

The technique we have used on soybeans is essentially identical to that described by Sullivan (1972), except that injury is induced by a 15-min incubation in a thermostatted water bath at about 50 C. Since the injury versus temperature curve is sigmoid, the sensitivity of the technique is greatest at temperatures inducing about 50% injury. We have found that on the average a 15-min incubation at 50 C will usually result in about 50% injury, but slight adjustments ($\pm$ 1 C) in the incubation temperature during the growing season may be necessary to compensate for the acclimatization or "hardening" of soybean plants that occurs as the summer progresses.

During the summer of 1975, we used the heat injury test to evaluate the heat tolerance of 15 varieties and 10 advanced selections. Each of these 25 strains were replicated four times in 4-row, 20-foot plots. Moisture stress during and after planting (May 13) resulted in poor germination and subsequent uneven and reduced stands. Normal temperatures and rainfall occurred during June, but with the exception of some rainfall on July 19, hot and dry weather conditions prevailed throughout July, August, and September. Heat injury determinations were performed on each plot at three different dates (July 7, July 24, and Aug. 6). Heat injury data for the 15 varieties are shown below:

Entry	% heat injury (avg. 3 dates)*	Entry	% heat injury (avg. 3 dates)*
Corsoy	36.7	Wayne	53.9
Harcor	39.5	Hark	54.2
Pomona	43.1	Clark 63	54.8
Calland	46.4	Kent	55.8
Woodworth	47.4	Cutler 71	57.7
Wells	50.1	Beeson	59.1
Williams	51.6	Bonus	63.6
Amsoy 71	52.8		

*L.S.D. (0.05) = 7.5.

Highly significant varietal differences in heat injury were observed on each sampling date and on the combined average across sampling dates. These varietal differences in heat injury were, for the most part, relatively consistent over sampling dates. The variety 'Bonus,' for example, ranked highest in heat injury on the last two sampling dates and second highest on the first sampling date. Similarly, 'Beeson' consistently ranked high in heat injury on each sampling date. At the other end of the spectrum, 'Corsoy' and 'Harcor' consistently ranked low in heat injury. It is interesting to note that Corsoy and Harcor possess leaves that are darker green, somewhat smaller, and more fibrous than those of Bonus and Beeson. Very low yields were obtained from this trial due to the reduced stands mentioned earlier and to the protracted drought occurring from mid-season on. Generally the early-maturing varieties yielded more than the late-maturing varieties. Correlations of yield with heat injury were negative as expected. However, only the correlation for the third sampling date was significant. Correlations of heat injury with maturity were nonsignificant.

We are continuing our investigations of this technique to determine if heat injury is a reliable indication of heat tolerance. Some morphological characteristics related to the degree of heat injury are also being studied.

References

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2) Stomatal frequency and heat and drought stress in soybeans.

Stomata are the primary anatomical structures involved in water loss and gas exchange in plants. Genetic differences in stomatal frequency may have potential in breeding for increased photosynthetic or water-use efficiency. Miskin and Rasmusson (1970), for example , found that barley lines with low stomatal frequencies transpired less water than lines with high stomatal frequencies, with no observed difference in the rates of photosynthesis. In another study, Ciha and Brun (1975) reported differences in stomatal frequencies among soybean varieties and suggested the possibility of genetically controlling water loss without affecting photosynthesis by reducing stomatal frequency. These authors expressed stomatal frequency as the number of stomata per square mm of leaf.

In 1974, a study was conducted to determine differences in stomatal frequency among soybean varieties. The relationship of stomatal frequency to heat and drought tolerance was also investigated. Six genotypes, three heat tolerant and three intolerant, as measured by a temperature-mediated leaf injury technique (Sullivan, 1972), were grown as part of a heat and drought tolerance study involving 24 strains and varieties. Each genotype was replicated four times in 20-foot, 4-row plots in a split-plot arrangement with irrigated and nonirrigated conditions representing the whole plots.

Five plants per plot, selected at random, were sampled for each of the six genotypes. The acrylic imprint method of Brown and Rosenberg (1970) was used to determine stomatal frequency. Acrylic imprints were made on a portion of the abaxial side of the terminal leaflet of the top, fully expanded trifoliolate leaf, at a point midway between the base and the tip. The imprints were lifted from the leaf with cellophane (Scotch 600) tape and attached to microscope slides. The slides were placed under a microscope equipped with a model "T" type camera from which the lens had been removed, so that the image was projected onto the camera back (normally covered by film). Thus, the camera back served as a screen for the enlarged image, allowing the stomata and epidermal cells to be counted without eyestrain or photography. Stomatal frequency for each genotype was expressed as a stomatal index: $I = [(\text{stomata})/(\text{stomata} + \text{epidermal cells})]\,100$. This formulation, first suggested by Salisbury (1927), expresses stomatal frequency as a ratio of stomata to the total number of epidermal cells, and eliminates the effects of epidermal cell size that could influence stomatal frequency measurements based on number per unit area.

The results (Table 1) show highly significant differences in the stomatal indexes among the six genotypes observed. Differences in stomatal indexes for varieties under different water treatments were not significant. Stomatal index showed no correlation to heat tolerance under nonirrigated conditions, but under irrigation, heat tolerant genotypes tended to have a lower stomatal index.

References

Brown, K. W. and N. J. Rosenberg. 1970. Influence of leaf age, illumination, and upper and lower surface differences on stomatal resistance of sugar beet (Beta vulgaris) leaves. Agron. J. 62: 20-24.

Ciha, A. J. and W. A. Brun. 1975. Stomatal size and frequency in soybeans. Crop Sci. 15: 309-312.

Table 1

Stomatal indexes of six soybean genotypes

Genotype	Stomatal index						
	Irrigated	Nonirrigated	$\overline{x}$				
Hark	10.99	10.36	10.68	a*			
Chippewa 64	8.94	10.84	9.87	a	b		
Beeson	9.97	9.18	9.57	a	b	c	
A66-1746-9	8.22	10.53	9.38	a	b	c	d
Bonus	9.42	8.13	8.78		b	c	d
L66-1359	8.33	6.92	7.63				d
Means	9.31	9.33	9.32				

*Values followed by the same letter are not significantly different at the .05 level.

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1) Multiple resistance to insects.

Soybean lines PI 229.358, PI 171.451 and PI 227.687 were reported to be resistant to Mexican bean beetle (*Epilachna varivestis*) (Van Duyn, Turnipseed